# Methylglyoxal Comes of AGE

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**The posttranslational modification of proteins by methylglyoxal, a highly reactive compound derived from glycolysis, may contribute to aging, diabetes, and other disorders. In this issue of *Cell*, Brownlee and colleagues (Yao et al., 2006) demonstrate a specific mechanism by which methylglyoxal modifies a transcriptional corepressor to enhance gene expression.**

Methylglyoxal is a highly reactive α-oxoaldehyde that is formed in cells primarily from the triose phosphate intermediates of glycolysis, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. In diabetes, hyperglycemia triggers enhanced production of methylglyoxal, one consequence of which is the rapid modification of proteins and other substrates to generate what are called *a*dvanced *g*lycation *e*nd products, or AGEs (Figure 1; Shinohara et al., 1998). In this issue of *Cell*, Brownlee and colleagues (Yao et al., 2006) propose a mechanism by which methylglyoxal modification of the corepressor protein mSin3A leads to an increase in the expression of angiopoietin-2. This provides an elegant example of how this common modification may regulate gene expression and could contribute to complications in diabetic patients.

Yao et al. (2006) studied Müller glia cells of the rat retina and show that glycolytic flux driven by high concentrations of glucose resulted in increased methylglyoxal modification of the corepressor protein mSin3A. The principal targets of methylglyoxal modification are arginine residues, yielding end products such as argpyrimidine and hydroimidazolone. Exquisite studies performed by Yao et al. (2006) identified the culprit arginine residues within the paired amphipathic helix 4 (PAH4) domain of mSin3A. This modification enhanced recruitment of *O*-β-N-acetylglucosaminyltransferase (OGT) to a complex of mSin3A and the transcription factor Sp3, which consequently increased modification of Sp3 by *O*-linked N-acetylglucosamine. These critical events diminished the binding of the repressor complex to a glucose-responsive GC box in the *angiopoietin-2* promoter, thereby increasing expression of the *angiopoietin-2* gene. Molecules such as angiopoietin-2 and other proangiogenic factors are key contributors to proliferative changes that characterize the advanced stages of diabetic retinopathy. Thus, Yao et al. (2006) describe new roles for methylglyoxal and glycation in the regulation of gene expression in Müller cells in the context of diabetes and the regulation of angiopoietin-2 expression. However, these findings suggest potential roles for methylglyoxal in the modulation of gene expression in other tissues that are susceptible to the complications of diabetes and in other contexts, such as cancer, renal failure, aging, infection, and inflammation.

It is well established that AGEs may exert multiple and diverse effects in diabetes and other pathological conditions. AGEs may result in the crosslinking of structural and basement membranes, thereby modifying key cellular structures that impact permeability and cellular motility. In addition, AGEs may transduce signals via their interaction with the cell-surface receptor RAGE (receptor for AGE). Through this interaction, AGEs upregulate production of a host of inflammatory molecules and molecules provoking tissue injury, which contributes to the development of accelerated atherosclerosis and microvascular complications in diabetes such as nephropathy (Ramasamy et al., 2005).

AGEs also may modulate interactions between cells. AGEs have been found on lymphocytes, raising the question of whether these modifications are biomarkers of the aging or diabetic state or whether they alter antigen presentation and T cell responses (Poggioli et al., 2002). Recent studies suggest that glycation may promote dendritic cell development but impair the ability of these cells to stimulate primary T cell responses (Price et al., 2004). It is plausible that modulation of cognate dendritic cell-T cell responses by AGE modifications may redirect immune responses in AGE-enriched environments. Based on the evidence that AGEs derived from methylglyoxal or other sources trigger maladaptive responses in multiple tissues in diabetes or aging, blockers of AGE production and stimulators of AGE degradation have been developed and are being tested in clinical trials.

The enzyme glyoxalase I is a critical defense against glycation in vivo (Thornalley 1993). Glyoxalase I detoxifies reactive α-oxoaldehydes, thereby removing deleterious species such as methylglyoxal. However, depletion of glutathione, as observed in settings of oxidative stress, suppresses activity of glyoxalase I. One consequence of oxidative stress is decreased glutathione, which could lead to enhanced accumulation of methylglyoxal and methylglyoxal-derived AGEs via decreased activity of glyoxalase I. Also, evidence sug-

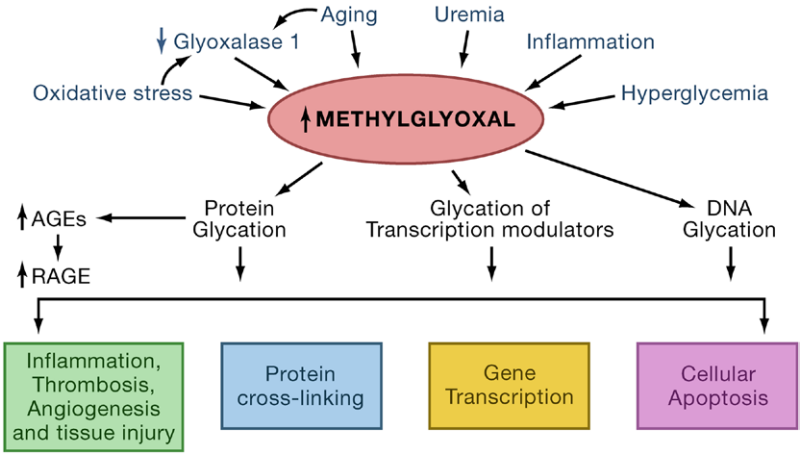


**Figure 1. Methylglyoxal: Changing the Face of the Proteome and the Genome**
Many factors contribute to the accumulation of methylglyoxal, including hyperglycemia, uremia, oxidative stress, aging, and inflammation. Methylglyoxal can react with and modify both proteins and DNA, leading to the generation of *a*dvanced *g*lycation *e*nd products (AGEs). The modification of targets by methylglyoxal and its derivatives contributes to upregulation of inflammatory and tissue-injury-provoking molecules (at least in part through the interaction of AGEs with their receptor RAGE), gene transcription, protein crosslinking, and apoptosis. In this issue of *Cell*, Brownlee and colleagues (Yao et al., 2006) report a new role for methylglyoxal in the modulation of gene expression through glycation of critical arginine residues in the corepressor protein mSin3A. Understanding the biology of methylglyoxal may shed light on conditions and processes as diverse as diabetes, uremia, cancer, and aging.

gests that the generation of reactive oxygen species is increased in diabetes (Nishikawa et al., 2000) and that AGEs themselves may generate reactive oxygen species. The interaction of AGEs with RAGE results in oxidative stress via activation of NADPH oxidase and mitochondrial pathways (Ramasamy et al., 2005). Also, the modification of antioxidant proteins by methylglyoxal may diminish their function, thereby further magnifying the generation of reactive oxygen species. Thus, unless the cycle is broken, conditions favoring oxidative stress may synergize with hyperglycemia, uremia (the retention of waste products in the blood due to renal failure), and aging to propel sustained methylglyoxal generation. In the studies by Brownlee and colleagues (Yao et al., 2006), the authors provide critical evidence linking oxidative stress and methylglyoxal-induced modification of mSin3A. Specifically, in addition to overexpression of glyoxalase I in retinal Müller cells, overexpression of uncoupling protein-1 (UCP-1) or manganese superoxide dismutase (MnSOD) prevented methylglyoxal-induced modification of mSin3A.

The studies of Brownlee and colleagues (Yao et al., 2006) also highlight exciting possibilities for cancer research. Certainly the role of angiopoeitin-2 extends beyond Müller cells and pathological angiogenesis in the glucose-laden retina, considering the critical biological importance of angiogenesis in cancer. The next step is to examine whether and how methylglyoxal modification of transcription factors contributes to oncogenesis (perhaps by regulating genes involved in metastasis or angiogenesis). In addition to protein substrates, methylglyoxal may glycate DNA (Thornalley, 2003). The presence of nucleotide AGEs in DNA may be associated with increased frequency of mutations, DNA strand breaks, and cytotoxicity. In the uremic environment, DNA glycation may increase the incidence of malignancy. In the tumor milieu, the consequences of glycation triggered by methylglyoxal modification of DNA may be exacerbated by concomitant protein glycation. For example, heat shock protein 27 (Hsp27) is a major target of methylglyoxal modification. This modification, which results in the formation of argypyrimidine-Hsp27, may repress cytochrome c-mediated caspase activation (Sakamoto et al., 2002).

However, other evidence suggests that methylglyoxal accumulation could also promote the apoptosis of tumor cells. *S*-*p*-bromobenzylglutathione diesters have been developed that inhibit glyoxalase I activity. These compounds facilitate accumulation of methylglyoxal and induce apoptosis of tumor cells (Thornalley et al., 1996). These experiments strongly suggest that the substrates of glyoxalase I have potent antitumor activity. Thus, at multiple stages in tumorigenesis and tumor progression, methylglyoxal-triggered glycation may leave its mark. Although in certain biological contexts, suppression of methylglyoxal and methylglyoxal-modified species may help to prevent or treat disease, in other settings, harnessing the potent power of methylglyoxal may fill a critical void in tumor therapy, the development of agents that stimulate tumor cell apoptosis.

Taken together, the findings of Brownlee and colleagues add to the growing body of evidence suggesting that methylglyoxal is more than a biomarker of disease and aging (see Figure 1). Furthermore, the new work highlights that, in addition to direct glycation of nucleotides with its associated consequences, the indirect impact of methylglyoxal on gene expression through glycation of critical residues in corepressors such as mSin3A is striking. Thus, the authors have identified a new link between glycation of protein residues and the modulation of gene expression. These findings highlight the power of methylglyoxal to change the face of the proteome and the genome.

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# DNA Double-Strand Break Repair: A Relentless Hunt Uncovers New Prey

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**A major pathway for repair of DNA double-strand breaks is nonhomologous end-joining (NHEJ). In this issue of *Cell*, Buck et al. (2006a) and Ahnesorg et al. (2006) report the discovery of a new NHEJ factor called Cernunnos-XLF. Both groups report that this protein is mutated in a rare inherited human syndrome characterized by severe immunodeficiency, developmental delay, and hypersensitivity to agents that cause DNA double-strand breaks.**

Double-strand breaks (DSBs) are a dangerous form of DNA damage. Unrepaired or misrepaired DNA ends can cause detrimental outcomes for cells and organisms, including cell death, chromosomal instability, and neoplastic transformation (Mills et al., 2003). These catastrophic lesions are generated during normal metabolic processes such as DNA replication or upon exposure to exogenous agents such as ionizing radiation or certain chemotherapeutic compounds. Several pathways exist that recognize and repair these lesions, including the nonhomologous end-joining (NHEJ) pathway, which serves to protect and directly ligate broken ends (Haber, 2000). Remarkably, despite the inherent risks, there are examples throughout nature where organisms have evolved systems to intentionally induce DSBs. These processes usually function to increase diversity of species or somatic cells by initiating the rearrangement of DNA at specific regions of the genome. An incredible example of this is V(D)J recombination, which occurs during B and T lymphocyte development to generate the vast diversity of antigen receptor genes that form the basis of the adaptive arm of our immune system. Although this process is initiated by lymphoid-specific factors, the rearrangements are completed by the ubiquitously expressed NHEJ components (Rooney et al., 2004).

To date, six NHEJ factors have been discovered: Ku70, Ku80, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Artemis, XRCC4 and DNA Ligase IV. Ku70 and Ku80 comprise a heterodimer that binds to DNA ends and recruits DNA-PKcs, a serine/threonine protein kinase. DNA-PKcs forms a functional complex with Artemis, which possesses an intrinsic single-strand 5′ to 3′ exonuclease activity. DNA-PKcs phosphorylates and activates the endonuclease activity of Artemis, allowing this protein to cleave DNA hairpins and other structures containing single- to double-stranded transitions. Thus, Artemis provides an important nucleolytic processing activity to prepare DNA ends for ligation. The NHEJ ligation activity is provided by Ligase IV in complex with the XRCC4 cofactor. Together, these six proteins possess the major activities required for NHEJ, which suggested that all members of the pathway had been identified.

Genes encoding two of the six factors, *Artemis* and *Ligase IV*, have been